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A new species of *Rhacophorus* (Anura, Rhacophoridae) from Xizang, China, with a revision of the distribution of *R. bipunctatus*

Shiyang Weng[1*], Xiaolong Liu[2,3*], Jianchuan Li[4], Guohua Yu[5,6], Junkai Huang[3]

1 *Institute of Plateau Biology of Xizang, Lhasa, 850030, China*
2 *Integrative Science Center of Germplasm Creation in Western China (CHONGQING) Science City, Biological Science Research Center, Southwest University, Chongqing, 400715, China*
3 *Key Laboratory of Freshwater Fish Reproduction and Development (Ministry of Education), School of Life Sciences, Southwest University, Chongqing, 400715, China*
4 *Xizang Museum of Natural Science, Lhasa, 850011, China*
5 *Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection (Guangxi Normal University), Ministry of Education, Guilin, 541004, China*
6 *Guangxi Key Laboratory of Rare and Endangered Animal Ecology, College of Life Science, Guangxi Normal University, Guilin, 541004, China*

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Corresponding authors: Junkai Huang (1198094210@qq.com); Guohua Yu (yugh2018@126.com)

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Abstract

A new species of *Rhacophorus* (Anura, Rhacophoridae), *Rhacophorus medogensis* **sp. nov.**, is described from Medog, Xizang Tibetan Autonomous Region, China. The new taxon can be distinguished from all phylogenetically closely related taxa (*R. rhodopus* and *R. bipunctatus* complexes) by the following combination of features: 1) body size medium (adult males SVL 31.6–38.7 mm, n = 17; adult females SVL 50.1–55.7 mm, n = 2); 2) pineal ocellus obvious; 3) toe webbing formula: I1–1II1–1.5III1–1IV1–1V; 4) irregularly shaped large black spots, white pattern in black spots on flanks; 5) snout pointed with appendage on tip; 6) tongue pyriform, with a deep notch at posterior tip; 7) tibiotarsal articulation reaching eye. In addition, we also discuss the distribution of *R. bipunctatus*, which is limited to northern India and central-western Myanmar, rather than the traditionally presumed range across South and Southeast Asia.

Key Words

Biodiversity, cryptic species, *Rhacophorus medogensis* sp. nov., taxonomy

Introduction

The genus *Rhacophorus* Kuhl & Van Hasselt, 1822, currently includes 46 species and is widely distributed across the tropical and subtropical regions of southern Asia, from India to China and Southeast Asia (AmphibiaChina 2024; AmphibiaWeb 2024; Frost 2024). *Rhacophorus bipunctatus*, commonly known as the Himalaya Flying Frog, was first described from the Khasi Hills in northern India (Bordoloi et al. 2007). The species has been reported from a broad distribution across South and Southeast Asia, including Bangladesh, Cambodia, China, Laos, Malaysia, Myanmar, Thailand, and Vietnam (Frost 2024). However, the taxonomic relationship between *R. bipunctatus* and *R. rhodopus* has been a topic of considerable debate over recent decades (Inger et al. 1999; Frost et al. 2006; Bordoloi et al. 2007; Chan et al. 2018).

* These authors contributed equally to this work.

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Recent phylogenetic analyses have revealed the complexity in the relationships between *Rhacophorus bipunctatus* and *R. rhodopus*. Chan et al. (2018) suggested that they belong to distinct species complexes, indicating potential cryptic diversity. The phylogenetic analyses of Che et al. (2020) showed that the "*R. rhodopus*" population from Medog, Xizang, is distantly related to the type locality of *R. rhodopus* and is morphologically more similar to *R. bipunctatus* (Mathew & Sen, 2010), classifying it as *R. bipunctatus*. However, Li et al. (2022) and Tang et al. (2024) showed that the "*R. bipunctatus*" from Medog is distantly related to *R. bipunctatus* from northern India; four species (*R. rhodopus*, *R. napoensis*, *R. qiongica*, and *R. kio*) in China belong to the *R. rhodopus* and *R. bipunctatus* complexes. However, these studies did not include molecular and morphological data from the type locality of *R. bipunctatus*, leaving uncertainties regarding the true distribution and diversity within this complex. To address these gaps, this study incorporated sequences from near the type locality of *R. bipunctatus* (Mawblang, Cherapunji, Southern Khasi Hills, northern India) to reconstruct the phylogenetic relationships within the *R. rhodopus* and *R. bipunctatus* complexes. Based on both morphological and molecular evidence, we also describe a new species of *Rhacophorus* from Medog, Xizang.

Materials and methods sampling

Fieldwork was conducted at Medog, Xizang Tibetan Autonomous Region, China. Eight specimens were collected in May 2024. The specimens were collected by hand and subsequently euthanized with a low concentration of clove oil solution following standard euthanasia protocols for amphibians (Leary et al. 2020). Liver or muscle tissues were taken from the specimens and preserved in 95% ethanol. While the specimens were fixed in 75% ethanol. Voucher specimens SWU 0008599, SWU 0008600, SWU 0008601, SWU 0008602, SWU 0008603, SWU 0008604, SWU 0008699, and SWU 0008701 were deposited at Southwest University (SWU).

Morphology and morphometrics

We measured all the voucher specimens. All the measurements were made with slide calipers to the nearest 0.1 mm. Morphological terminology followed Fei (1999) and Fei et al. (2009) and is listed in Table 1; the webbing formula followed Myers and Duellman (1982). Morphological measurements of all specimens are listed in Table 2. Comparative morphological data of the new species and congeneric species were obtained from published literatures (Liu and Hu 1960; Ohler and Delorme 2006; Bordoloi et al. 2007; Fei et al. 2009, 2012; Chan and Grismer 2010; Rowley et al. 2012; Matsui et al. 2013; Li et al. 2022; Tang et al. 2024; Che et al. 2020). Sex of the specimens was determined by the presence of nuptial pads and vocal sac openings.

Table 1. Morphological characters used for adult individuals.

Abbreviation	Morphology
SVL	Snout-vent length
HL	Head length
HW	Head width
SL	Snout length
IND	Internarial distance
IOD	Interorbital distance
UEW	Width of upper eyelid
ED	Diameter of eye
TD	Diameter of tympanum
DNE	Distance from nostril to eye
FHL	Length of forearm and hand
TL	Tibia length
TFL	Length of foot and tarsus
FL	Foot length

DNA sequencing and molecular analyses

To construct a phylogeny for *Rhacophorus rhodopus* and *R. bipunctatus* complexes, we extracted total DNA from liver or muscle tissue using the Animal Tissue DNA Isolation Kit provided by Thermo Fisher Scientific. In this study, we chose five specimens (SWU 0008599, SWU 0008600, SWU 0008601, SWU 0008603, and SWU 0008699) to sequence three consecutive mitochondrial gene segments: partial 12S rRNA, $tRNA^{val}$, and partial 16S rRNA. The primers used for polymerase chain reaction (PCR) amplification are detailed in Table 3. The PCR amplification process was carried out in a 50-µL reaction volume, following the reaction cycling settings below: an initial denaturing step at 95 °C for 4 min; 35 cycles of denaturing at 94 °C for 60 s, annealing at 51 °C for 60 s, and extending at 72 °C for 60 s; and a final extending step of 72 °C for 10 min. Sequencing was conducted using the corresponding PCR primers and an internal primer (Rhint: 5'-GACAGTGTAACCCTCGTGAT-3') (Yu et al. 2019), and all sequences have been uploaded to GenBank (Table 4). Based on Tang et al. (2024), we selected species from the genera *Rhacophorus*, *Buergeria*, *Nyctixalus*, *Chiromantis*, *Theloderma*, *Kurixalus*, *Zhangixalus*, and *Leptomantis* as outgroups to reconstruct the phylogenetic relationships of the *R. rhodopus* and *R. bipunctatus* complexes; all GenBank accession numbers are listed in Table 4.

Sequences were aligned using the MUSCLE option in MEGA v. 7, and due to the absence of 12S rRNA and $tRNA^{val}$ sequences in some species, uncorrected pairwise distances (p-distances) were calculated only for 16S rRNA sequences (500 bp) between species (Kumar et al. 2016). Prior to phylogenetic reconstruction, the best substitution model was selected using the Akaike Information Criterion (AIC) in jMODELTEST v. 2.1.10 (Darriba et al. 2012). Bayesian inference (BI) was performed using MrBayes v.3.2.6 (Ronquist et al. 2012), with two runs performed simultaneously, each consisting of four Markov chains starting from a random tree. The chain was run for 6,000,000 generations, with sampling every

Table 2. Measurements (mm) of adult specimens in the type series of *R. medogensis* sp. nov.

NO	SWU 0008699	SWU 0008599	SWU 0008600	SWU 0008601	SWU 0008602	SWU 0008603	SWU 0008604	SWU 0008701
SEX	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male	Subadult
SVL	33.5	36.2	34.1	35.4	33.5	34.0	34.6	27.4
HL	12.1	13.4	11.8	13.1	12.0	12.3	12.7	10.8
HW	11.1	12.2	11.5	12.4	11.2	11.6	12.0	10.4
SL	5.0	5.4	5.3	5.4	5.3	5.2	5.3	4.7
IND	3.2	3.7	3.1	3.5	3.5	3.4	3.3	2.7
IOD	4.6	4.5	4.6	4.6	4.8	4.5	4.7	3.6
UEW	2.5	2.7	2.9	2.8	2.7	2.5	2.7	2.2
ED	4.2	4.6	4.3	4.4	4.4	4.2	4.4	3.6
TD	2.2	2.3	2.1	2.2	2.2	1.9	2.1	1.9
DNE	2.6	2.7	2.4	2.4	2.4	2.6	2.5	2.2
FHL	16.3	16.5	16.5	17.1	16.8	16.9	18.3	13.9
TL	16.6	17.9	16.3	17.7	16.2	17.6	17.6	14.5
TFL	22.4	23.0	21.9	23.5	22.7	23.3	23.3	18.4
FL	14.0	14.8	13.2	14.9	14.5	15.2	15.1	11.9

Table 3. Primer pairs for PCR amplification used in this study.

Gene	Primer	Source
12S rRNA, $tRNA^{val}$, and partial 16S rRNA	L1091 (5′–AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT–3′)	Kocher et al. 1989
	16H1 (5′–CTCCGGTCTGAACTCAGATCACGTAGG–3′)	Hedges 1994

1,000 generations. When the average standard deviation of the split frequency was less than 0.01, the first 25% of the sampled trees were discarded as burn-in, and the remaining trees were used to create a consensus tree and estimate the Bayesian posterior probabilities. Maximum likelihood (ML) analyses were performed using RAxML v7.0.3 (Stamatakis 2014) under the GTRGAMMA model with 1000 bootstrap replicates.

Results

Phylogenetic analyses

The obtained sequence alignment was 1936 bp long. The phylogenetic trees inferred from BI and ML methods are generally consistent (Fig. 1). The phylogenetic results of this study show differences in the placement of certain species compared to Tang et al. (2024) but consistently indicate that the *Rhacophorus bipunctatus* complex is nested within the *R. rhodopus* complex. The *R. rhodopus* and *R. bipunctatus* complexes comprise 10 known species and 1 undescribed lineage (*R. medogensis* sp. nov., *R. rhodopus*, *R. napoensis*, *R. qiongica*, *R. borneensis*, *R. norhayatiae*, *R. reinwardtii*, *R. bipunctatus*, *R. kio*, *R. helenae*, and *R.* sp.). The sequence of *R. bipunctatus* from the near-type locality clusters together with those from northeastern India and central-western Myanmar, representing the "true" *R. bipunctatus*. The new species *R. medogensis* sp. nov. clusters within the *R. rhodopus* and *R. bipunctatus* complexes and represents the sister group to *R. borneensis*, *R. norhayatiae*, and *R. reinwardtii* with strong support (BPP = 0.98, BS = 77). The undescribed lineage (*R.* sp.) represents the sister group to *R. rhodopus* with strong support (BPP = 0.99, BS = 95). P-distances of 16S rRNA between the sequences of *R. medogensis* sp. nov. and the other species of *R. rhodopus* and *R. bipunctatus* complexes varied from 7.5% to 11.6%, while the p-distances of 16S rRNA between *R.* sp. and *R. rhodopus* is 7% (Table 5).

Taxonomic account

Rhacophorus medogensis sp. nov.

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Figs 3, 4, Table 2

Chresonymy. *Rhacophorus rhodopus*—Hu 1987; Fei et al. 2004; Fei et al. 2009; Li et al. 2011; Fei et al. 2012; Li et al. 2022.

Rhacophorus bipunctatus—Che et al. 2020; Tang et al. 2024

Holotype. SWU 0008699, adult male, collected in May 2024 by Xiaolong Liu, Renda Ai, and Xianqi Li from Didong Village, Medog, Xizang Tibetan Autonomous Region, China (29.2205°N, 95.1293°E, elevation 771 m; Fig. 2A).

Paratypes. Six adult males (SWU 0008599, SWU 0008600, SWU 0008601, SWU 0008602, SWU 0008603, and SWU 0008604) were collected at Buqun (Xigong) Lake, Medog, Xizang Tibetan Autonomous Region, China (29.25241°N, 95.225759°E, elevation 1361 m). One subadult (SWU 0008701) was collected at the same locality as the holotype in May 2024 by Xiaolong Liu, Renda Ai, and Xianqi Li.

Table 4. Species used in phylogenetic analyses of this study.

No.	Species	Voucher	Locality	Accession No
1	*R. medogensis* sp. nov.	KIZ016380	Medog, Xizang, China	MW111517
2	*R. medogensis* sp. nov.	YPX40427	Medog, Xizang, China	MW111518
3	*R. medogensis* sp. nov.	L06245	Medog, Xizang, China	JX219441
4	*R. medogensis* sp. nov.	L062456	Medog, Xizang, China	JX219442
5	*R. medogensis* sp. nov.	SWU 0008599	Medog, Xizang, China	PQ963460
6	*R. medogensis* sp. nov.	SWU 0008600	Medog, Xizang, China	PQ963459
7	*R. medogensis* sp. nov.	SWU 0008601	Medog, Xizang, China	PQ963458
8	*R. medogensis* sp. nov.	SWU 0008603	Medog, Xizang, China	PQ963457
9	*R. medogensis* sp. nov.	SWU 0008699	Medog, Xizang, China	PQ963456
10	*R. bipunctatus*	CAS229913	Nagmung Township, Putao District, Kachin State, Myanmar	JX219445
11	*R. bipunctatus*	CAS235303	Mindat Township, Mindat District, Chin State, Myanmar	JX219444
12	*R. bipunctatus*	PUCZM/IX/SL360	Mizoram, Inida	MH087073
13	*R. bipunctatus*	PUCZM/IX/SL612	Mizoram, Inida	MH087076
14	*R. bipunctatus*	/	Mawblang, Cherapunji, Southern Khasi Hills, India*	OL988889
15	*R. napoensis*	GXNU YU000171	Napo, Guangxi, China	ON217796
16	*R. napoensis*	GXNU YU000173	Napo, Guangxi, China	ON217798
17	*R. napoensis*	VNMN:4118	Yen Tu, Bac Giang, Vietnam	LC010605
18	*R. napoensis*	VNMN:4120	Pu Huong, Nghe An, Vietnam	LC010609
19	*R. napoensis*	VNMN:4121	Thanh Hoa, Vietnam	LC010608
20	*R. napoensis*	AMNH-A 161418	Huon Son Reserve, Ha Tinh, Vietnam	AY843750
21	*R. qiongica*	SN 030035	Hainan, China	EU215529
22	*R. qiongica*	VNMN:4117	K' Bang, Gia Lai, Vietnam	LC010604
23	*R. qiongica*	FMNH253114	Ankhe Dist, Gia Lai, Vietnam	GQ204716
24	*R. rhodopus*	clone 5	Mengyang, Yunnan, China	EF646366
25	*R. rhodopus*	SCUM 060692L	Mengyang, Yunnan, China	EU215531
26	*R. rhodopus*	KIZ060821229	Lvchun, Yunnan, China	EF564574
27	*R. rhodopus*	2004.0409	Long Nai Khao, Phongsali, Laos	KR828049
28	*R. rhodopus*	2006.2519	Ban Vang Thong, Louangphrabang, Laos	KR828069
29	*R. rhodopus*	K3046	Doi Chiang Dao, Chiang Mai, Thailand	KR828066
30	*R. rhodopus*	K3085_1	Mae Lao-Mae Sae Wildlife Sanctuary, Chiang Mai, Thailand	KR828067
31	*R.* sp	clone 4	Jingdong, Yunnan, China	EF646365
32	*R.* sp	KIZ060821248	Jingdong, Yunnan, China	EF564575
33	*R.* sp	KIZ060821175	Yongde, Yunnan, China	EF564573
34	*R.* sp	clone 2	Yongde, Yunnan, China	EF646363
35	*R.* sp	KIZ587	Longling, Yunnan, China	EF564577
36	*R.* sp	KIZ589	Longling, Yunnan, China	EF564578
37	*R.* sp	USNM:Herp:587063	Kandawgyi National Gardens, Mandalay, Myanmar	MG935991
38	*R.* sp	0937Y4	Kui Buri NP, Prachuap Khiri Khan, Thailand	KR828058
39	*R.* sp	KUHE:53375	Genting, Pahang, Malaysia	LC010569
40	*R. borneensis*	NMBE 1056517	Batang Ai NP, Sarawak, Malaysia	JN377366
41	*R. borneensis*	BORN 22411	Sabah, Maliau Basin, Malaysia	AB781694
42	*R. helenae*	UNS 00451	Binh Thuan, Vietnam	JQ288090
43	*R. kio*	KUHE 55165	Xuan Lien, Than Hoa, Vietnam	AB781695
44	*R. lateralis*	/	Mudigere, India	AB530548
45	*R. norhayatiae*	Rao081205	Malaysia	JX219443
46	*R. norhayatiae*	NNRn	Johor, Endau Rompin, Malaysia	AB728191
47	*R. nigropalmatus*	Rao081204	Malaysia	JX219437
48	*R. reinwardtii*	ENS 16179 (UTA)	Java, Patuha, Indonesia	KY886328
49	*Buergeria buergeri*	TTU-R-11759	Japan	AF458122
50	*Chiromantis rufescens*	CAS 207601	Bioko Norte Province, Equatorial Guinea	AF458126
51	*Kurixalus idiootocus*	CAS 211366	Taipei, Taiwan, China	AF458129
52	*Leptomantis gauni*	FMNH 273928	Bintulu, Sarawak, Malaysia	JX219456
53	*Nyctixalus pictus*	FMNH 231094	Lahad Datu, Sabah, Malaysia	AF458135
54	*Theloderma albopunctatum*	ROM 30246	Vietnam	AF458148
55	*Zhangixalus smaragdinus*	HM05292	Xima, Yingjiang, Yunnan, China	MN613221

Etymology. The specific epithet "*medogensis*" is named after the type locality, Medog, Xizang, China. We suggest "Xizang flying frog" as its English common name and "Mò Tuō Shù Wā" (墨脱树蛙) as its Chinese common name.

Diagnosis. The genus *Rhacophorus*, also known as flying frogs, is characterized by the following features: 1) body size relatively moderate or large (SVL 30–100 mm, above 40 mm in most species); 2) presence of intercalary cartilage between terminal and penultimate phalanges of digits; 3)

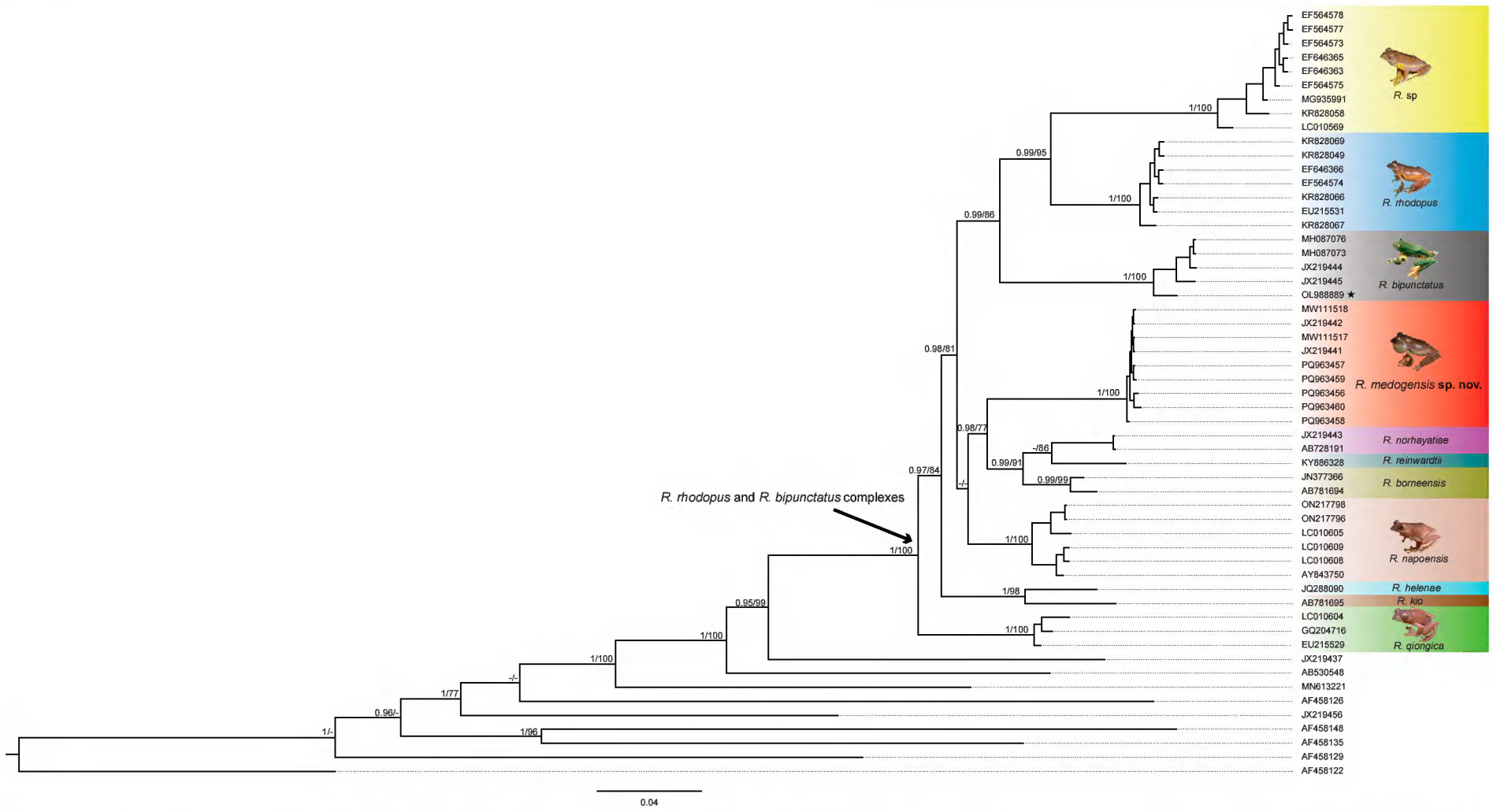

Figure 1. Bayesian phylogenetic tree of *R. rhodopus* and *R. bipunctatus* complexes and related species inferred from 12S rRNA, tRNA-^{Val}, and 16S rRNA genes. The numbers above and below the branches are Bayesian posterior probabilities (BPP) and maximum likelihood bootstrap values (BS), "-" denotes a BPP < 0.95 and BS < 70. The scale bar represents 0.04 nucleotide substitutions per site. The "black star" means this sequence from from near type locality of *R. bipunctatus*, *R. medogensis* sp. nov., *R. rhodopus* and *R.* sp photoed by Xiaolong Liu, *R. bipunctatus* from Bordoloi et al. (2007), *R. qiongica* photoed by Chenxi Liao, *R. napoensis* by Junkai Huang.

Table 5. Mean uncorrected pairwise distances (%) between clades of *R. rhodopus* and *R. bipunctatus* complexes and related species based on 16S rRNA sequences.

No	Species	1	2	3	4	5	6	7	8	9	10	11
1	*R. medogensis* sp. nov.											
2	*R. bipunctatus*	10.3										
3	*R. napoensis*	8.4	8.8									
4	*R.* sp	9.7	9.7	10.4								
5	*R. qiongica*	9.1	13.1	10.7	13.9							
6	*R. reinwardtii*	8.6	9.2	7.3	9.3	12.2						
7	*R. rhodopus*	11.6	10.1	11.3	7.0	12.3	10.3					
8	*R. norhayatiae*	8.6	9.5	8.1	9.3	13.5	5.8	11.1				
9	*R. helenae*	10.3	10.1	9.9	10.4	12.4	8.9	13.2	10.1			
10	*R. borneensis*	7.5	8.7	6.7	8.6	10.1	6.4	11.7	5.7	6.7		
11	*R. kio*	10.4	11.1	9.5	11.3	13.5	11.2	14.0	9.3	6.8	7.8	

terminal phalanges of fingers and toes Y-shaped; 4) tips of the digits expanded into large disks bearing circummarginal grooves; 5) webbed fingers; 6) skin not co-ossified to the skull; 7) upper eyelid projections absent, tarsal projections present in most species; 8) dermal folds along the forearm or tarsus present; 9) pupil horizontal; 10) iris without "X"-shaped marking; 11) white foam nests or jelly-encapsulated eggs produced by breeding pairs; and (12) distributed mainly in Indochina (Jiang et al. 2019). *Rhacophorus medogensis* sp. nov. is placed in the genus *Rhacophorus* due to the combination of the following features: 1) body size relatively moderate (adult males SVL 31.6–38.7 mm, n = 17; adult females SVL 50.1–55.7 mm, n = 2); 2) presence of intercalary cartilage between terminal and penultimate phalanges of digits; 3) terminal phalanges of fingers and toes Y-shaped; 4) tips of the digits expanded into large disks bearing circummarginal grooves; 5) webbed fingers; 6) tarsal projections present; 7) pupil horizontal; 8) iris without "X"-shaped marking. *Rhacophorus medogensis* sp. nov. can be distinguished from other species in the *R. rhodopus* and *R. bipunctatus* complexes by the following combination of features: 1) medium adult males body size (adult males SVL 31.6–38.7 mm); 2) dorsal surface reddish brown, light green, light brown, or grayish green in life; 3) pineal ocellus obvious; 4) toe webbing formula: I1–1II1–1.5III1–1IV1–1V; 5) irregularly shaped large black spots, white pattern in black spots on flanks; 6) snout pointed with an appendage on the tip; 7) tongue pyriform, with a deep notch at the posterior tip; 8) throat rough; 9) palm rough with small tubercles; 10) tibiotarsal articulation reaching the eye.

Figure 2. (A) Large habitat at the type locality of *R. medogensis* sp. nov., Didong Village, Medog County, Xizang Tibetan Autonomous Region, China (29.2205°N, 95.1293°E, elevation 771 m); (B) macrohabitat of *R. medogensis* sp. nov. (SWU 0008601), Buqun (Xigong) Lake, Medog County, Xizang Tibetan Autonomous Region, China (29.25241°N, 95.225759°E, elevation 1361 m).

Description of holotype. Adult male, medium body size (SVL 33.5 mm); head length (HL 12.1 mm) longer than head width (HW 11.1 mm); snout pointed with an appendage on tip, sloping in profile, and protruding beyond the margin of lower jaw in ventral view; snout length (SL 5.0 mm) is longer than the diameter of the eye (ED 4.2 mm); the canthus rostralis is distinct and curved; loreal region oblique, concave; nostrils oval, lateral, slightly protuberant, and slightly closer to the tip of snout than the eye; the internarial space (IND 3.2 mm) is slightly smaller than the interorbital distance (IOD 4.6 mm) and larger than the width of the upper eyelid (UEW 2.5 mm); the pupil is horizontal; pineal ocellus obvious; tympanum distinct (TD 2.2 mm), rounded, and nearly about half of eye diameter (ED 4.2 mm); the supratympanic fold is narrow and flat; tongue pyriform, with a deep notch at the posterior tip; choanae oval; vomerine teeth present in two series; with an internal single subgular vocal sac; a vocal sac opening on the floor of the mouth at each corner (Fig. 3).

Forelimbs strong, length of forearm and hand (FHL 16.3 mm); relative length of fingers I < II < IV < III; tips of all fingers expand into discs with circummarginal and transverse ventral grooves, disc of finger I smaller than discs of other fingers; entire web between fingers, webbing formula: I2–2II1–1.5III1–1IV; subarticular tubercles rounded and prominent, formula 1, 3, 4, 4; inner metacarpal tubercle single, oval, and prominent (Fig. 3C).

Hindlimbs slender and long, heels overlapping when legs at a right angle to the body, tibiotarsal articulation reaching the eye; tibia length (TL 16.6 mm) nearly equal to the length of forearm and hand (FHL 16.3 mm), longer than foot length (FL 14.0 mm), and shorter than the length of tarsus and foot (TFL 22.4 mm); relative length of toes I < II < III < V < IV, with the third and fifth toes being nearly equal in length; tips of all toes expanded into discs with circummarginal and transverse ventral grooves; entire web between toes, webbing formula: I1–1II1–1.5III1–1IV1–1V; subarticular tubercles rounded and prominent, formula 1, 1, 2, 3, 2; supernumerary tubercles absent; single inner metatarsal tubercle, oval, outer metatarsal tubercle absent; tibiotarsal joint with a small triangular fold of skin (Fig. 3D).

Dorsal skin smooth with very fine granules; throat rough, covered with small warts, and ventral surface of forelimbs smooth; palm rough with small tubercles; chest, belly, and ventral surface of small warts (Fig. 3A, B); dermal folds on forearm, tarsus, heels, and vent present.

Coloration of holotype in life. For coloration of the holotype in life (see Fig. 4). Dorsal surface reddish brown with a dark brown X-shaped marking; dark brown patches between the eyes; the dorsal surface of the body and limbs covered with small black dots; the supratympanic fold reddish brown; iris orange brown; the dorsal surface of limbs has distinct dark brown bands; a larger black spot present between the axillary and inguinal regions, as well as several smaller black spots located between the two larger spots, some white patterns in the black spots on flanks; ventral surface creamy white; throat pale yellow; ventral surface of the limbs orange; finger webbing yellow and toe webbing reddish orange; both the finger and toe discs are yellow.

Coloration of the holotype in preservative. After preservation in alcohol, the color faded, but the general pattern did not change. Dorsal color changed to grey-brown with a dark X-shaped marking and some small black dots; the ventral surface faded to white; the white pattern in the large black spots on the flanks has disappeared (Fig. 3A, B).

Sexual dimorphism. Males are smaller than females (Che et al. 2020); the forearms are slightly more robust, with a milky-white nuptial pad on the inner side of the first finger base; they possess a single internal subgular vocal sac, with oval-shaped openings that are relatively large.

Variation. The coloration in living individuals is variable. The dorsal surface typically displays a reddish-brown coloration, characterized by irregular dark brown or chestnut spots. Some individuals present light green, light yellow, or light brown coloration. The dorsal coloration can change in response to variations in environmental colors and the individual's condition. The black spots on the flanks exhibit three morphological forms: 1) a larger black spot typically present in the axillary and inguinal regions (SWU 0008602); 2) several smaller black spots located between the two larger spots (SWU 0008599,

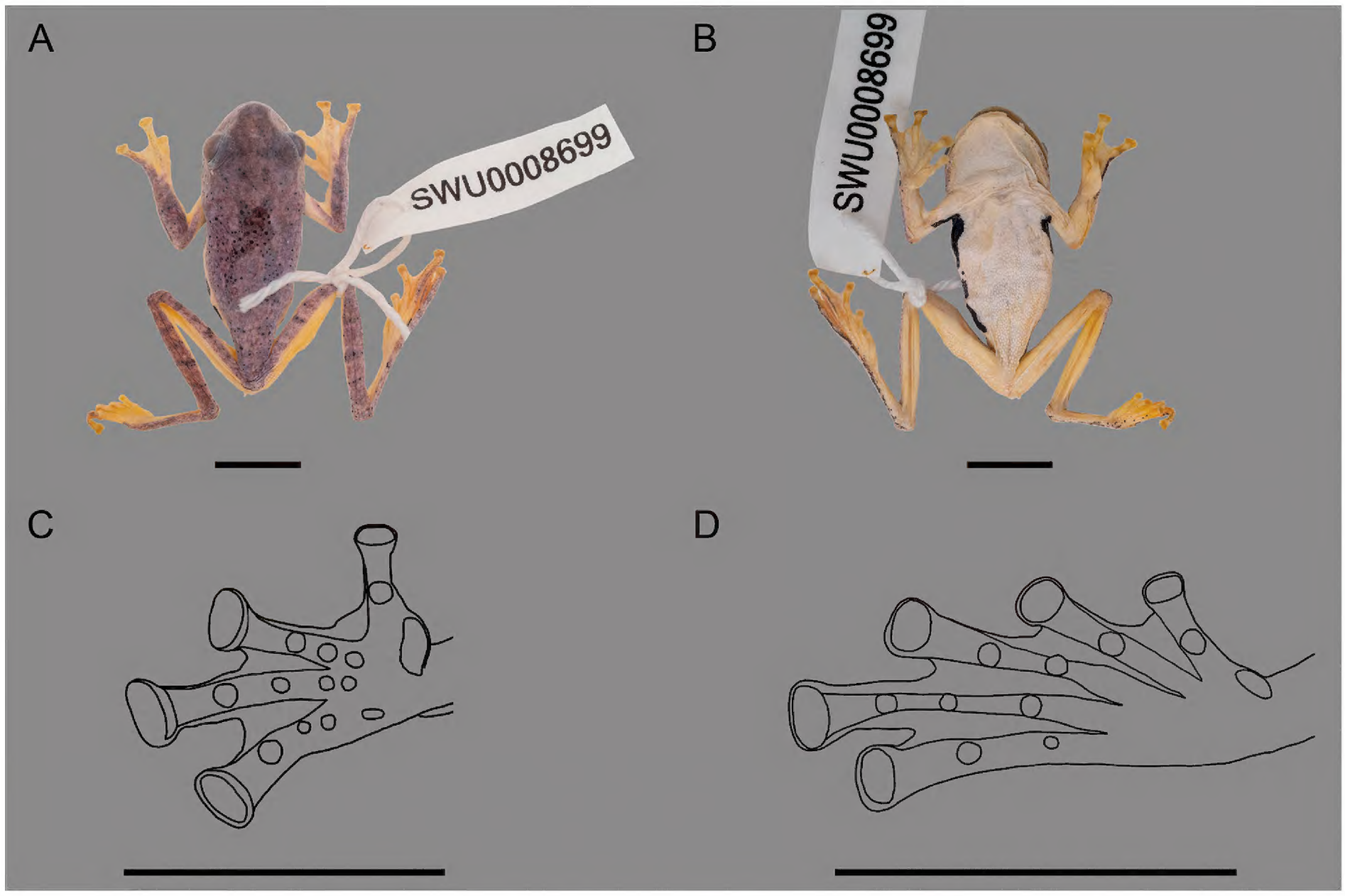

Figure 3. Holotype (SWU 0008699) of *R. medogensis* sp. nov. in preservative, showing (A) dorsal view, (B) ventral view, (C) Line drawing of the ventral view of hand, and (D) line drawing of the ventral view of foot. Scale bars: 10 mm.

SWU 0008600, SWU 0008601, and SWU 0008699); 3) the presence of a single axillary spot without an inguinal spot (SWU 0008604 and SWU 0008701).

Distribution and ecology. This species is currently known to be distributed only in Medog (Fig. 5). It inhabits humid shrublands, marshes, puddles, or slow-flowing ditches at elevations of 500–1700 m (Fig. 2B). Some individuals were found during the day in bamboo tubes with *R. tuberculatus*. Females are less common, and none were observed during surveys conducted in May.

Comparisons. Rather than comparing the new species to all known *Rhacophorus*, we focus on our morphological comparison with phylogenetically closely related taxa (*R. rhodopus* and *R. bipunctatus* complexes) (Table 6). *Rhacophorus medogensis* sp. nov. can be easily distinguished from *R. helenae*, *R. kio*, *R. borneensis*, *R. norhayatiae*, and *R. reinwardtii* by the dorsal surface being reddish brown (vs. green) and the web between toes being red with no black pigmentation (vs. black pigmentation present).

Rhacophorus medogensis sp. nov. differs from *R. bipunctatus* by 1) smaller body size (adult male SVL 31.6–38.7 mm, n = 17 vs. 37.8–50.4 mm, n = 28; Table 6); 2) dorsal surface reddish brown, light green, light brown, or grayish green in life (vs. dorsal surface green in life); 3) dorsal surface reddish brown, light green, light brown, or grayish green in life (vs. dorsal surface green in life); 4) white pattern in black spots on flanks (vs. blue pattern in black spots on flanks); 5) snout pointed with appendage on tip (vs. snout pointed without appendage on tip); 6) distinct bands on limbs (vs. indistinct bands on limbs); 7) palm rough with small tubercles (vs. palm smooth without small tubercles); 8) tibiotarsal articulation reaching eye (vs. tibiotarsal articulation reaching beyond eye); 9) tongue pyriform (vs. tongue rounded); 10) pineal ocellus obvious (vs. pineal ocellus absent).

Figure 4. Holotype (SWU 0008699) of *R. medogensis* sp. nov. in situ.

Rhacophorus medogensis sp. nov. differs from *R. napoensis* by 1) tibiotarsal articulation reaching eye (vs. tibiotarsal articulation reaching beyond eye); 2) tongue pyriform (vs. tongue cordiform); 3) pineal ocellus obvious (vs. pineal ocellus absent).

Table 6. Comparison of *R. medogensis* sp. nov. with species in *R. rhodopus* and *R. bipunctatus* complexes, "/" means unknown.

Species	*R. medogensis* sp. nov.	*R. bipunctatus*	*R. napoensis*	*R. qiongica*	*R. rhodopus*
Adult male	31.6–38.7 mm, n = 17	37.8–50.4 mm, n = 28	39.7–44.2 mm, n = 5	35.1–38.2 mm, n = 8	33.1–38.7 mm, n = 6
Adult female	50.1–55.7 mm, n = 2	37.3–59.1 mm, n = 8	/	49.3 mm, n = 1	50.2 mm, n = 1
Dorsal surface	Red brown, light green, light brown, or grayish green	Green	Red brown	Red brown	Red brown or yellow brown
Black spots on flanks	Two large spots in the axillary and inguinal regions	Two large spots	Two small spots	A series of small spots	One small spot in the axillary region
Pattern in black spots	White	Blue	/	Absent	/
Snout pointed	With appendage	Without appendage	With appendage	Without appendage	Without appendage
Bands on limbs	Distinct	Indistinct	Distinct	Distinct	Distinct
Throat	Rough	Rough	Rough	Smooth	Smooth
Palm	Rough	Smooth	Rough	Smooth	Rough
Tibiotarsal articulation	Reaching eye	Reaching beyond eye	Reaching beyond eye	Reaching beyond eye	Reaching eye
Tongue	Tongue pyriform	Tongue rounded	Tongue cordiform	Tongue cordiform	Tongue narrow and long
Pineal ocellus	Obvious	Absent	Absent	Absent	Absent
Toe webbing formula	I1–1II1–1.5III1–1IV1–1V	/	/	I1–1II1–1III1–1IV1–1V	/

Species	*R. kio*	*R. helenae*	*R. borneensis*	*R. norhayatiae*	*R. reinwardtii*
Adult male	58–79.1 mm, n = 10	72.3–85.5 mm, n = 3	50.9 mm, n = 1	60.6–64.7 mm, n = 5	41.7–49.8 mm, n = 6
Adult female	/	89.4–90.7 mm, n = 2	62.0 mm, n = 1	75.7–83 mm, n = 4	66.6–74.8 mm, n = 2
Dorsal surface	Green	Green	Green	Green	Green
Black spots on flanks	One large spot in the axillary region	One large spot in the axillary region	Irregular and mainly concentrated in the axillary region	Irregular in the axillary and inguinal regions	One spot in the axillary region
Pattern in black spots	Absent	Absent	Dark blue	Blue	Orange or yellow
Snout pointed	Without appendage	Without appendage	Without appendage	Without appendage	Without appendage
Bands on limbs	Distinct	Indistinct	Absent	Absent	Absent
Throat	Smooth	Smooth	/	/	/
Palm	Rough	/	/	/	/
Tibiotarsal articulation	Reaching beyond eye	/	Reaching point between eye and nostril	Not extend beyond snout	/
Tongue	Tongue moderate and oval	/	/	Tongue oval	/
Pineal ocellus	Absent	Absent	Absent	/	/
Toe webbing formula	I1–1II1–1III1–1IV1–1V	I1–1II1–1III1–1IV1–1V	I1–1II1–1III1–1IV1–1V	I1–1II1–1III1–1IV1–1V	I1–1II1–1III1–1IV1–1V

Rhacophorus medogensis sp. nov. differs from *R. qiongica* by 1) usually two large spots in the axillary and inguinal regions (vs. a series of small black spots on flanks); 2) white pattern in black spots on flanks (vs. no pattern in black spots on flanks); 3) snout pointed with appendage on tip (vs. snout pointed without appendage on tip); 4) throat rough (vs. throat smooth); 5) palm rough with small tubercles (vs. palm smooth without small tubercles); 6) tibiotarsal articulation reaching eye (vs. tibiotarsal articulation reaching beyond eye); 7) tongue pyriform (vs. tongue cordiform); 8) pineal ocellus obvious (vs. pineal ocellus absent); 9) toe webbing formula: I1–1II1–1.5III1–1IV1–1V (vs. toe webbing formula: I1–1II1–1III1–1IV1–1V).

Rhacophorus medogensis sp. nov. differs from its sister species *R. rhodopus* by 1) usually two large black spots in the axillary and inguinal regions (vs. one small black spot in the axillary region); 2) snout pointed with appendage on tip (vs. snout pointed without appendage on tip); 3) throat rough (vs. throat smooth); 4) tongue pyriform (vs. tongue narrow and long); 5) pineal ocellus obvious (vs. pineal ocellus absent).

Discussion

In this study, we reconstructed the phylogenetic relationships within the *Rhacophorus rhodopus* and *R. bipunctatus* complexes by including sequences from near the type locality of *R. bipunctatus* (Mawblang, Cherapunji, Southern Khasi Hills, northern India). Our results support previous studies indicating that *R. bipunctatus* and *R. rhodopus* are not monophyletic and likely represent multiple cryptic lineages (Chan et al. 2018; Che et al. 2020; Tang et al. 2024). The inclusion of samples from near the type locality allowed us to

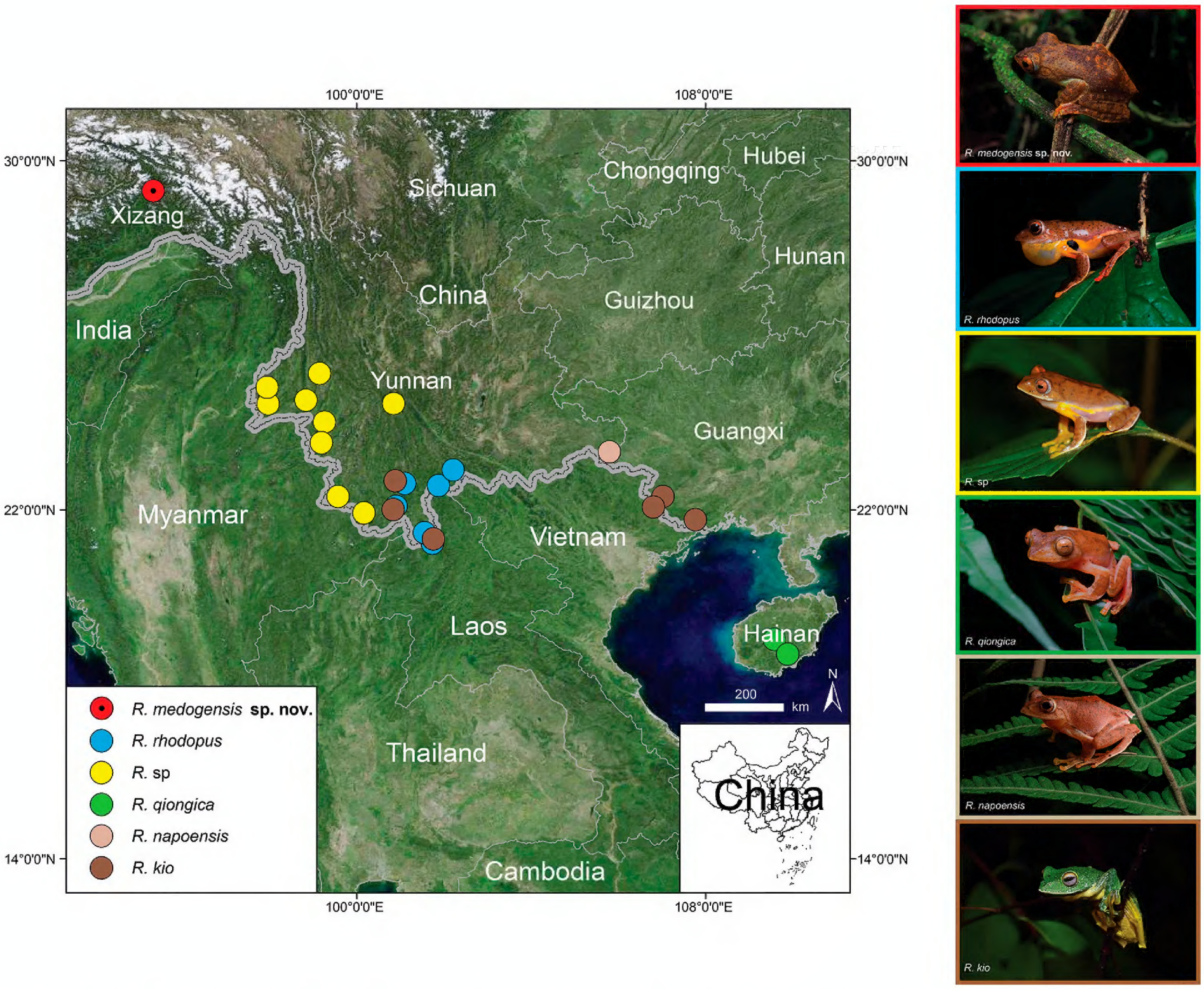

Figure 5. Map showing the distribution pattern of *R. rhodopus* complex species in China. Distribution sites accessed from Tang et al. (2024) and our data, *R. medogensis* sp. nov., *R. rhodopus* and *R.* sp photoed by Xiaolong Liu, *R. qiongica* photoed by Chenxi Liao, *R. napoensis* and *R. kio* photoed by Junkai Huang.

refine the distribution range of *R. bipunctatus*. Our phylogenetic analysis reveals that *R. bipunctatus* samples from near the type locality only cluster with those from central-western Myanmar, supporting the conclusion of Tang et al. (2024) that *R. bipunctatus* is limited to northern India and central-western Myanmar, rather than the traditionally presumed range across South and Southeast Asia (Frost 2024). In addition, our phylogenetic analysis identified a distinct cryptic species within the *R. rhodopus* complex distributed across western Yunnan, Myanmar, Thailand, and Malaysia. This lineage has historically been misidentified as *R. rhodopus* or *R. bipunctatus*. Due to a lack of available specimens, we provisionally refer to this lineage as *R.* sp. and recommend further investigation to clarify its taxonomic status.

The taxonomic ambiguity surrounding the populations in Medog, Xizang, also emerged as a key issue. Historically, researchers have referred to the Medog population as either *Rhacophorus rhodopus* or *R. bipunctatus* (Hu 1987; Fei et al. 2004, 2009, 2012; Li et al. 2011; Che et al. 2020). However, our results suggest that this population does not form a monophyletic group with either *R. rhodopus* from Yunnan or *R. bipunctatus* from India. Consequently, we describe this population as a new species, *R. medogensis*, to reflect its distinct evolutionary lineage and unique morphology.

Our findings also prompt a re-evaluation of the distribution records for species of *Rhacophorus rhodopus* and *R. bipunctatus* complexes in China. Fei et al. (2004) suggested that *R. rhodopus* was distributed in northern Medog and "true" *R. bipunctatus* in the south (Rotung area), but Che et al. (2020) suggested that only *R. bipunctatus* is distributed in Medog. Due to the lack of confirmed specimens of *R. bipunctatus* from southern Medog, we recommend that *R. bipunctatus* be excluded from distribution records in China until further specimen-based evidence is available. Currently, there are five known species of the *R. rhodopus* and *R. bipunctatus* complexes along with one undescribed species (*R. medogensis* sp. nov., *R. rhodopus*, *R. napoensis*, *R. qiongica*, *R. kio*, and *R.* sp) distributed across Xizang, Yunnan, Hainan, and Guangxi (Fig. 5).

Medog, located south of the Himalayas in Xizang, is recognized as a biodiversity hotspot (Myers et al. 2000). Recent infrastructure development, including road construction, has facilitated increased field surveys and led to the discovery of new species (Che et al. 2020; Yu et al. 2024; Shi et al. 2020). However, such development also poses potential threats to biodiversity in this region. It is therefore critical to conduct further comprehensive surveys to gain a deeper understanding of species diversity and implement conservation measures that address these emerging threats.

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